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With Great Power Settings, Come Great Learning Opportunities: Applying Washburn's Equation to Laser Cutter Fabricated Paper Microfluidic Chips

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Abstract:

Paper microfluidics is a field of interest for applications such as DNA detection, diagnostic tools, and environmental pollution tracking. Previously, in the junior Bioengineering laboratory class at the University of Pennsylvania, the paper microfluidics lab module was conducted by printing and then baking a wax layer onto Whatman filter paper. Taking inspiration from an experiment testing Washburn flow in a paper channel shaped with a CO_2 laser to cut and seal edges, backed nitrocellulose paper and a VLS 3.60DT laser cutter were used to develop a new version of the lab module. The goal of this paper is to create a procedure for the paper microfluidics module with a laser cutter that could be scaled to function in Bioengineering classes of various sizes and skill levels. Incorporating updated technology, such as the laser cutter, allows students to use equipment available to them in makerspaces. It was found that the procedure not only maintained the integrity of Washburn assumptions but also was better received by students.

We developed a procedure using cameras, rulers, and video analysis software to track the path of the fluid front to determine its adherence to the Washburn equation. To demonstrate functionality, 31 paper channels were tested and analyzed for a power relationship with a 95% CI of [0.465, 0.481] ($\mu = 0.47$, $\sigma = 0.02$). This average power relationship closely follows the 0.5 expected from the Washburn relationship. A survey was used to make a comparison between classes of subsequent years, with one class (n=47) using a laser, while the other (n=12) used a wax printer for fabrication. All but two questions from the survey showed statistically significant differences in responses from the two groups. The most notable differences in survey responses for laser cutter usage were in the consistency of paper microfluidic fabrication (average 49.3% increase in strong agreement), understanding of practical application (average 43.7% increase in strong agreement), and overall frustration with the module (47.8% decrease in strong agreement). There were a few limitations encountered with our current experimental setup that could be addressed in future iterations of this lab module. Namely, the minimum feature size was not limited by the width of the laser beam, but rather by charring from the cutting process and the inherent transient period needed for Washburn-like flow. Some future directions for this lab entail the application of Darcy flow, characterization of flow from channels that intersect, as well as three-dimensional channels.

Introduction:

Paper microfluidics is a field of special interest to Bioengineering students because of its use in DNA detection, diagnostic tools, and environmental pollution tracking [1]. The need for rapid diagnostic tools was amplified by the COVID-19 pandemic on college campuses across the country. Paper microfluidic chips served as a low-cost diagnostic that could be used with minimal medical experience to detect COVID-19 through the targeting of SARS-CoV-2 antigens and nucleic acids [2]. The following laboratory module seeks to expose students to the process of both designing and fabricating microfluidic chips on their own.

<u>Learning Objectives:</u>

The paper microfluidics laboratory module was created with the goal of having students:

1. Fabricate their own paper microfluidic chips with a laser cutter by shaping channels.
2. Study and characterize the physics of one-dimensional fluid flow in paper channels.
3. Work with limited nitrocellulose paper to efficiently plan the use of resources.
4. Demonstrate understanding of paper microfluidic flow through designing a device with the following specifications:
    - Start with one inlet
    - End at two different regions
    - Incorporate a turn in at least one channel
    - Time difference between the two regions must be 60 seconds +/- 5 seconds
    - Use a maximum of 6mL of fluid in the reservoir

The specifications listed for the demonstration above will be referenced as the design challenge. This paper focuses on the development and implementation of a different fabrication method for the University of Pennsylvania's Bioengineering Lab Class for juniors. The updated fabrication technique utilizes a laser cutter to shape paper channels that students will use to characterize the physics of fluid flow in channels restricted to one dimension. The governing equation for capillary flow in porous materials is Washburn's equation, which relates the position of the fluid front in a one-dimensional channel to time. The velocity of a fluid in a one-dimensional porous matrix, like paper, through capillary action is described using Washburn's equation:

$$L^2 = \frac{\gamma * D * t}{4 * \mu}$$

where γ is the effective surface tension, μ is viscosity, D is pore diameter, and L is the distance the fluid front moved by time t [3]. This equation is used to describe fluid flow that follows "Washburn behavior" in a channel, which assumes flow in one direction inside a constant-width channel and a separated wet and dry region [4]. The lab module focuses on the relationship between L and t, while assuming γ, μ, and D are constants for the backed nitrocellulose paper used in this experiment. As such, students are asked to find the power relationship between L and t through running their own experiments and to create a channel of their own to meet the criteria of the design challenge. The challenge requires students to experimentally determine how turns affect their equations, and have two connected, but separate channels complete their flow in their respective paths at specified times.

There are a variety of methods to fabricate paper microfluidic channels to create a channel that restricts fluid flow to one dimension. These methods are categorized as either patterning or shaping paper channels. Patterning layers a hydrophobic barrier, such as wax, onto the paper in a manner that outlines the channel, whereas shaping paper channels can be done with cutting, embossing, or laser etching channels from the paper itself [1]. Previously, in the junior Bioengineering laboratory class, the paper microfluidics lab module was conducted by printing a wax layer onto Whatman filter paper and then using a hot plate to bake the wax layer into the paper fibers, thus patterning a straight channel with a hydrophobic barrier restricting fluid flow to one dimension. Though wax printing is a strong candidate for paper microfluidic chip fabrication, there were problems applying the technique to a classroom that were revealed in the learning laboratory. More specifically, wax printing fabrication problems were concentrated around the baking step in the process, where wax is heated to fully penetrate pores in the paper in a defined area around the channel. Baking required a high skill level, difficult to master in the time frame given to complete the design challenge. Students would frequently under-bake the channel, resulting in fluid leaks under the barrier and out of the channel; students could also over-bake the channel, resulting in wax penetrating and subsequently clogging the channel barrier which would restrict fluid flow. Besides problems related to baking, the wax printer experienced frequent paper jams which would halt the design process.

Taking inspiration from an experiment that cut shaped paper channels [3] with a CO_2 laser to seal the edges and test Washburn flow, we developed a procedure to shape backed nitrocellulose paper into straight channels using a VLS 3.5/60 laser cutter. This method creates a seal along the edges of the channel that follows the laser's path, constraining fluid flow to one dimension. The goal of this paper is to report the newly developed procedure for the paper microfluidics module with a laser cutter that could be scaled to function in Bioengineering classes of various sizes and skill levels. Incorporating updated technology, such as the laser cutter, allows students to use equipment available to them in makerspaces while at the same time leveraging the engineering principles and tools utilized in previous classes. It was found that the procedure not only maintained the integrity of Washburn assumptions but also was better received by students.

Methods

Developing procedure:

The following procedure was completed using these materials:

- Universal VLS 3.5/60DT Laser Cutter (50W)
- 1 sheet of Hi-FlowTM Plus Membrane 135 (Millipore Pt. No. HF13502XSS)
- Scrap piece of MDF
- Scrap piece of Acrylic
- Surgical tape
- Transfer pipette
- Double-Sided Film Tape
- Reusable Tray

The first step in creating the procedure for laser cutting paper microfluidic chips was determining the configuration for the VLS 3.5/60DT Laser Cutter that: (1) burned the edge of the paper enough to prevent leakage out of the paper channel, (2) did not drastically change effective channel width, and (3) when wetted, had a solvent front that consistently followed a Washburn pattern. Firstly, it was most important that the laser cut backed nitrocellulose paper prevented leakage, since any leaks from the channel would deviate from the one-dimensional channel flow assumption for Washburn's equation. To determine if the edges were burnt enough to create a seal, a 1:1 mixture of water and red food coloring was left in the channels for extended periods of time, confirming that it did not spread or leak outside of the paper. Channels were also visually inspected under a microscope, showing a dark layer surrounding the channel pores for their entire length, indicating a proper seal. Additionally, the effective channel width could be calculated by tracing the distance between the two burnt ends at different spots along the nitrocellulose paper. Burns that spanned a significant portion of the channel width created a pinched point in the channel that would restrict fluid flow and fail to meet the assumption of constant width for Washburn's equation. All laser cut channels had some uneven burning when looking under a microscope. However, slight variations in width did not create large deviations from Washburn's equation. Video analysis software was used to check whether the fluid front followed a path in the paper channels that could be described using Washburn's equation. From this experimentation, we found a laser configuration that would meet the goals of our procedure and adhere to the power relationship expected from Washburn's equation. Using all the previously mentioned methods for validation, it was determined that the following parameters were best suited for laser cutting paper microfluidic channels from backed nitrocellulose paper: Power = 10%, Speed = 20%, PPI = 800.

A procedure was created using cameras, rulers, and video analysis software to track the paths of multiple fluid fronts at a time and confirm Washburn flow for each. A phone camera was held by a ring stand about 1 foot above the table surface, with all five channels and a ruler in frame. Using a transfer pipette, a drop of dyed water is added to all channels individually, recording five different channels at once. Afterwards, each channel's fluid front was analyzed using software to track the path of the fluid front by plotting points in the middle of the channel and measuring position along the direction of fluid flow against time. The fluid front was tracked across time until it made contact with the catch at the end of the paper microfluidic chip. Figure 1 shows a representative log vs. log graph of the position vs. time that students use to determine their power relationship.

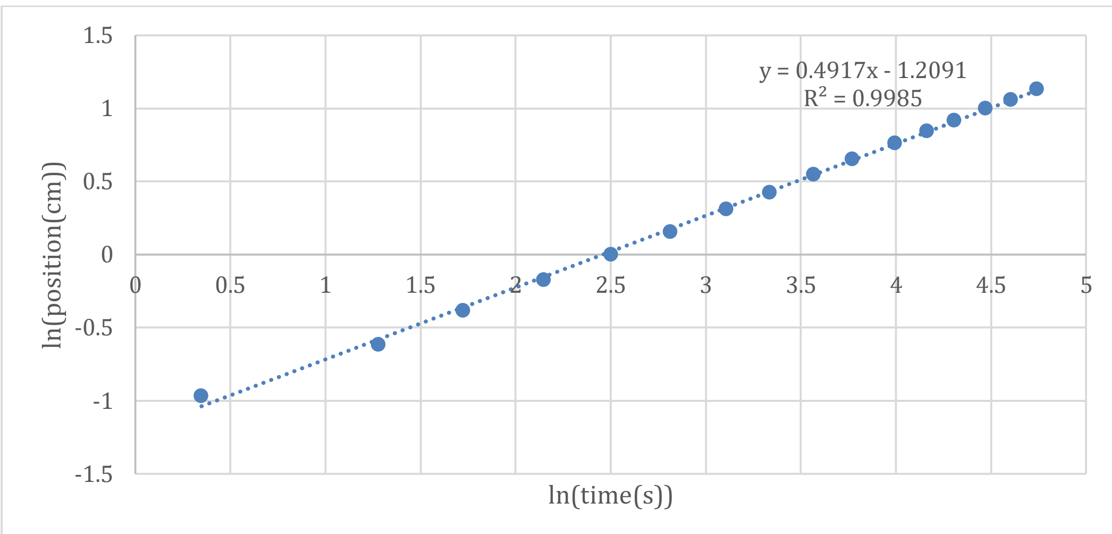

Figure 1: Representative log v. log graph students create equations from

When the lab module was first developed, it was ambiguous when to start recording the solvent front position against time. Through testing, it was observed that recording position against time as soon as a fluid front formed in a channel would not produce the expected power relationship of 0.5, suggesting that Washburn flow did not start immediately at the beginning of a straight paper channel. Instead, we observed a power relationship closer to the expected 0.5 when we started tracking time at the moment the fluid front appeared to be perpendicular to the direction of travel. Though a visual cue is used to determine the start of Washburn flow, this happened at about 2.54mm from the start of the channel on average (μ = 2.54mm, σ = 0.37mm).

The Lucas-Washburn equation describes flow through an open or closed channel with the assumption of a viscous flow regime with capillary forces counteracted by friction. Most importantly, the Lucas-Washburn Law assumes that inertial forces are negligible throughout the fluid flow being described. When fluid first flows into a channel, there are inertial forces at the beginning of capillary motion in a channel called the initial inertial regime that accelerate fluid flow. In this scenario, the friction from the wall surface area in contact with the fluid front is much smaller than the capillary force, creating an imbalance between these forces that causes flow to accelerate until friction balances with the capillary force. The initial inertial regime occurs in porous media like backed nitrocellulose paper and was considered when tracking the fluid front [5]. Though there is no way to visually confirm whether the flow is laminar for our experiment, the procedure attempted to account for this initial deviation from Washburn-like flow by waiting for the fluid front to appear more linear before recording position against time.

Student procedure:

The procedure in the lab manual given to students had several goals: to optimize the procedure for a laboratory class and to ease students into the tools they will use to fabricate these microfluidic chips of paper as they work towards a design challenge.

To streamline the procedure, students are trained on the laser cutter and provided with some materials at the start of the module, including a DWG file, which is a subset of a CAD (computer-aided design) file containing instructions for the laser cutter. The DWG file is used by students to set the 2D vector path that the laser will follow while cutting into the backed nitrocellulose paper. A 10" x 10" scrap piece of MDF, a 6" x 3" piece of acrylic, a DWG file as shown in figure 2, along with one sheet of backed nitrocellulose paper were provided to each student group. After going through laser cutter training in lab classes, students are prepared to use the laser cutter on MDF and acrylic. For the microfluidics lab, we introduce the concept of laser cutting paper to students for the first time; thus, we provide detailed instructions for their first few cuts. Using the VLS 3.5/60 laser cutter, students position the DWG paper channel file on the backed nitrocellulose paper while trying to conserve space and cut the channel out using the provided laser configurations. Students were then instructed to inspect the edges of the cut-out channel under a microscope to observe the seal created by the laser. Pore inspection of the paper channels helps demonstrate how the fluid will interact with the paper as it travels through the network of fibers. These observations will help students to confirm the assumptions of Washburn's Equation and understand why this is a necessary step for shaping a channel out of backed nitrocellulose paper.

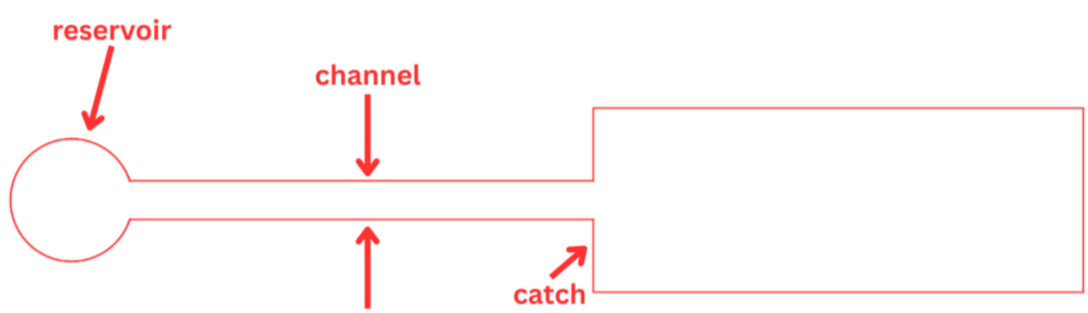

Figure 2: .DWG file given to students along with the anatomy of a paper microfluidic device

Each group is then instructed on how to run their first experiment using the provided acrylic slab and double-sided tape. One side of the double-sided tape is used to adhere the acrylic slab to a tray and provide grip for tape removal, while the other is used to both keep the paper channel flush and to store as many paper microfluidic channels as fit its width as shown in figure 3. This method reduces waste by reusing the acrylic slab between trials and maximizes the number of trials that can be recorded at once. Then, a phone is set up to record as a transfer pipette is used to add a drop of water to the circular reservoir, and it will record until the fluid front reaches the square catch. This video is then used to record the position of the fluid front only in the direction of fluid flow (one dimension) over time.

Figure 3: Photos from manual that show how students are to adhere their paper channels to the acrylic slab

As mentioned previously, the initial inertial regime present when fluid first enters the paper channel violates the assumptions of Washburn's law. Thus, students were made aware of when to start recording the position of the fluid front in a channel to account for the initial deviation from Washburn-like flow. The procedure directs groups to start measuring time when the fluid front becomes more linear in appearance. At this point, the distance from the start of the channel would be recorded, and time would start with t=0. This explanation in the procedure served as a visual cue to help students understand tracking better and helped bring the power relationship closer to 0.5 as expected.

The image found in Figure 4 is provided to students along with an explanation of what the points mean. The blue dashed line is drawn where point 1 is placed to indicate the start of the channel; this point is simply used to accurately track the total distance traveled. A red dashed line has been drawn on the channel where point 2 was placed, i.e., once the fluid front appeared flat in the second image. Point 2 is when time equals zero; however, distance does not equal zero.

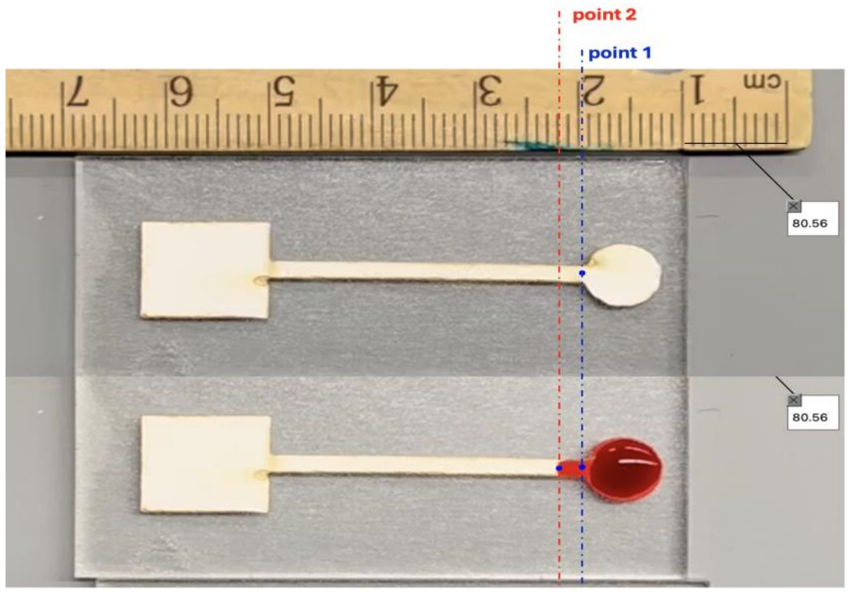

Figure 4: Annotated view of how students were instructed to plot position of the fluid front against time

Over the course of the next few lab periods, students will collect more trials and analyze those trials using video tracking software. After recording the position of the solvent front against time, students then fit a line to its log vs log graph to determine the power relationship between length and time. This will help each group develop their own Washburn equation describing flow in the backed nitrocellulose paper. Using the equation they find through analysis, the students will then finally design their own microfluidic chip. These chips must adhere to a couple of design constraints: (1) start with one inlet, (2) end at two different regions, (3) incorporate a turn into at least one channel, (4) have a time difference between the two regions of 60 seconds +/- 5 seconds, and (5) use a maximum of 6mL of fluid in reservoir. The point of including a turn is for students to model what happens when you start to deviate from the theoretical behavior and how to use experimental data to modify their equations to account for this deviation.

After students completed the module, a Qualtrics survey was created with questions to assess the students' understanding of paper microfluidics in theory and practice, and their attitudes towards the paper microfluidic channel module. After completing the course, an anonymous survey was distributed to the class who had just completed the module using the laser cutter, and a class who completed the module using a wax printer the previous year.

Results

As stated previously, the goal of this paper was to develop a new procedure that maintained functionality and obtained better responses from students in the Bioengineering curriculum. To demonstrate functionality, 31 functional paper microfluidic channels were cut using the laser cutter procedure and analyzed for a power relationship. The average power relationship had a 95% CI of [0.465, 0.481] ($\mu = 0.47$, $\sigma = 0.02$), which is close to the 0.5 expected from the Washburn relationship described previously.

A Qualtrics survey was used to make a comparison between the class of 2026 and the previous class year's opinions on the paper microfluidic module for their junior lab class (BE 3090). One class completed the lab module using a laser cutter, while the other class used a wax printer. There were 47 responses from students who used the laser cutter and 12 from the class that used a wax printer for fabrication. Responses were recorded using a Likert scale. Some of the most notable differences between the two modules for students were in the consistency of paper microfluidic fabrication and understanding of practical application. The survey consisted of twelve questions that were used to gauge students' interest in microfluidics along with their comfort in fabricating and predicting trends.

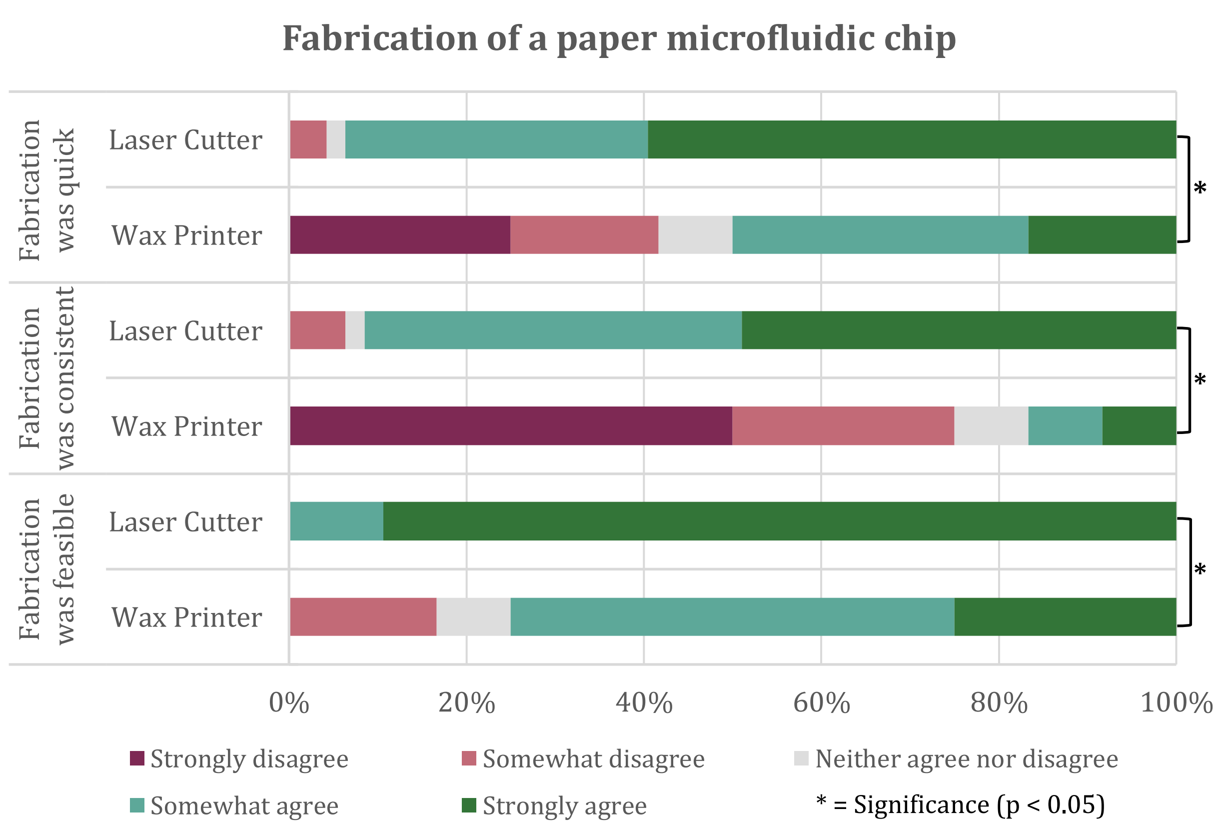

Figure 5: survey responses for questions regarding fabrication of paper microfluidic chips with either laser cutter (top) or wax printer (bottom)

Figure 5 displays the survey results for questions regarding the fabrication of a paper microfluidic chip from students that used wax printers compared to laser cutters for fabrication. About 5% of the class fabricating with a laser cutter disagreed with the statement that fabrication was consistent, compared to the over 70% who disagreed with that statement in the wax printing cohort. Running a Wilcoxon rank sum test revealed that there was a significant difference found in the responses to each survey question regarding the fabrication of paper microfluidic chips.

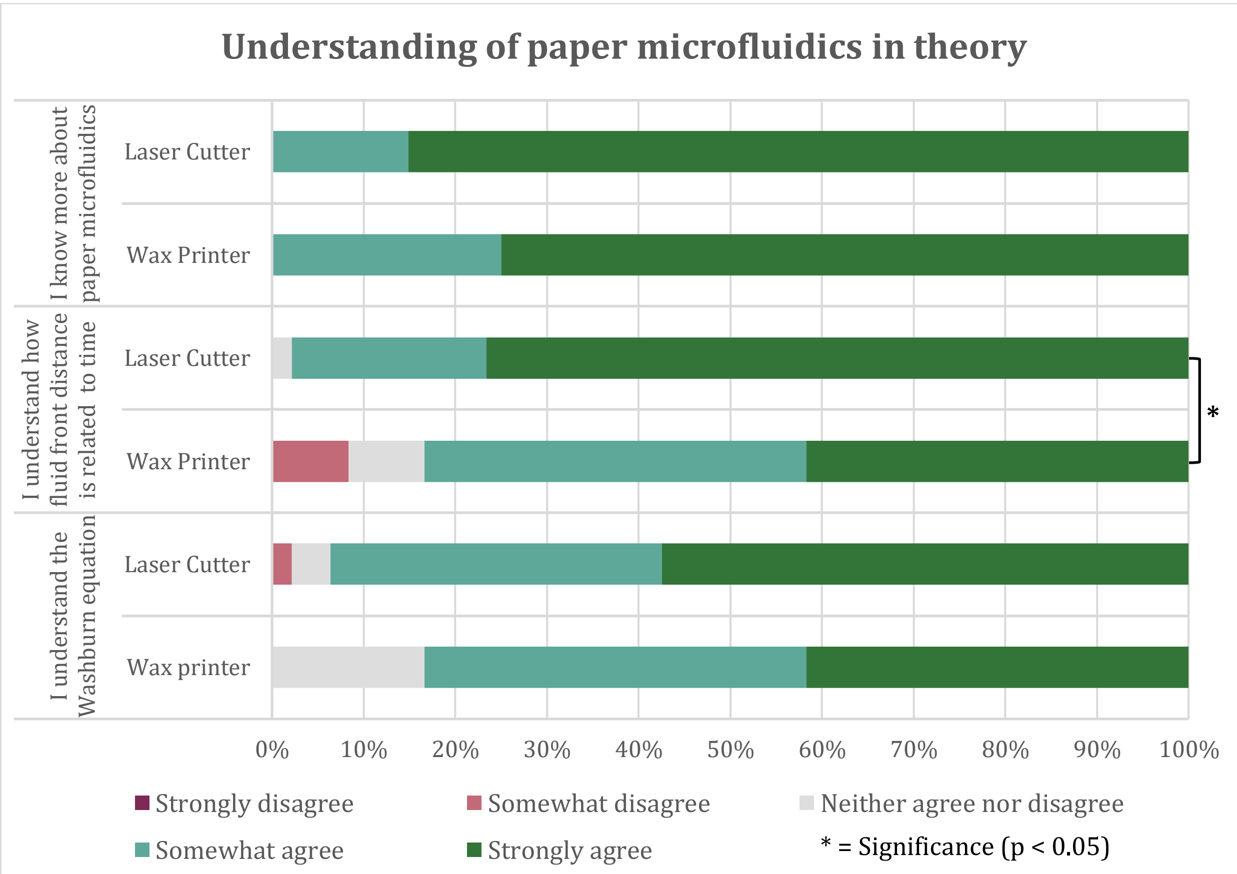

Figure 6: survey responses for questions regarding understanding of paper microfluidic theory (laser cutter responses = left, wax printer responses = right)

Figure 6 displays the survey results for questions regarding the theory of paper microfluidic chips from students that used wax printers compared to laser cutters for fabrication. It was observed that there were little differences in the responses between fabrication techniques. A Wilcoxon rank sum test revealed that there was only a significant difference found in the responses to the statement "I understand how fluid front distance is related to time". This data supports our goal of developing a new procedure that does not compromise student understanding.

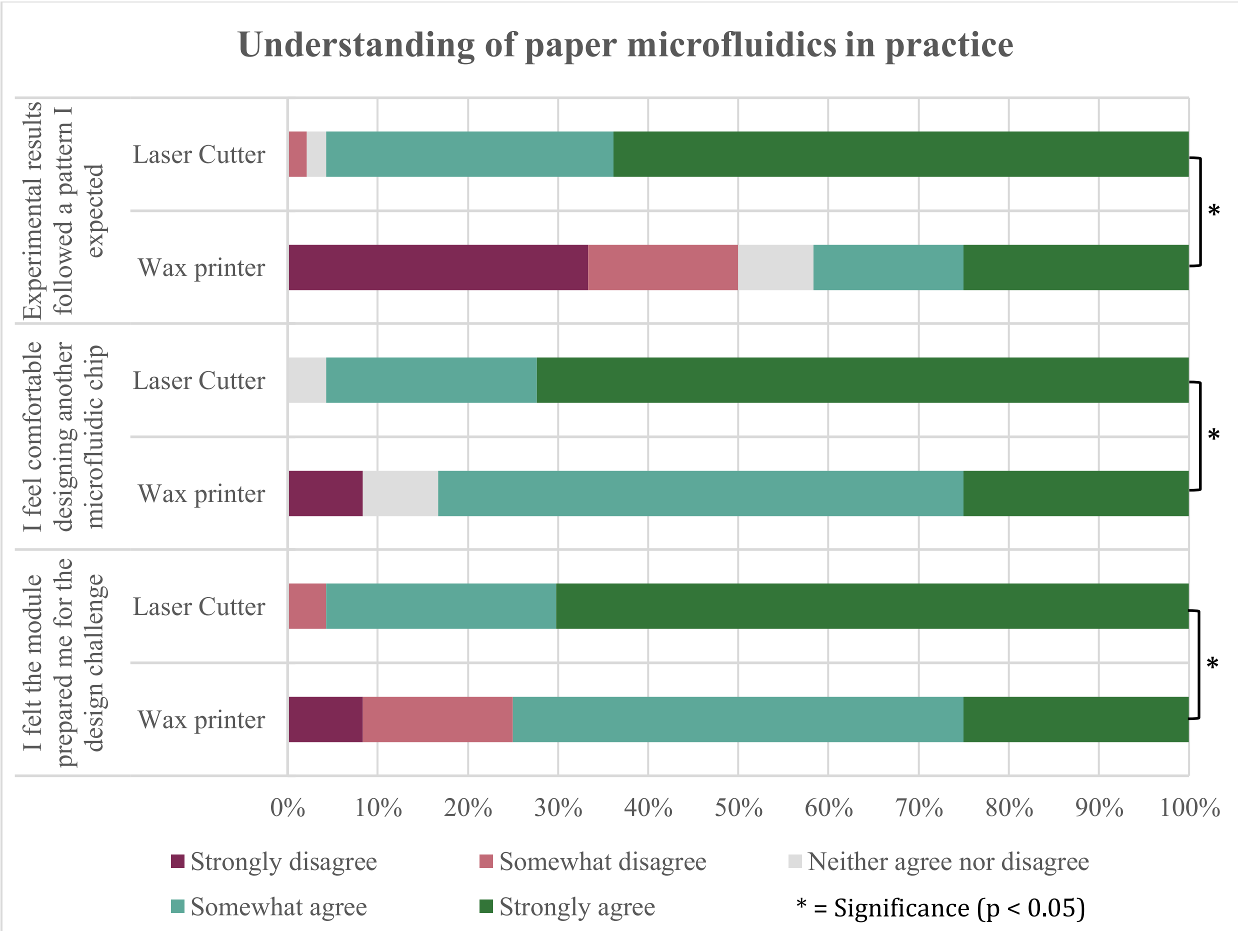

Figure 7: survey responses for questions regarding paper microfluidic chips in practice (laser cutter responses = left, wax printer responses = right)

Figure 7 displays the survey results for questions regarding the understanding of paper microfluidic chips in practice from students that used wax printers compared to laser cutters for fabrication. Perhaps the most important finding was that the microfluidic chip followed a behavior pattern that students recognized through their equations. In the section of the survey measuring understanding of paper microfluidics in practice, about 50% of the students who fabricated using a wax printer responded that they did not feel the experimental results followed a behavior pattern they expected. The percentage of students who disagreed with that statement dropped to less than 10% when using the laser cutter as their fabrication technique. The Wilcoxon rank sum test demonstrated a significant difference in the responses to each survey question regarding the understanding of paper microfluidic chips in practice.

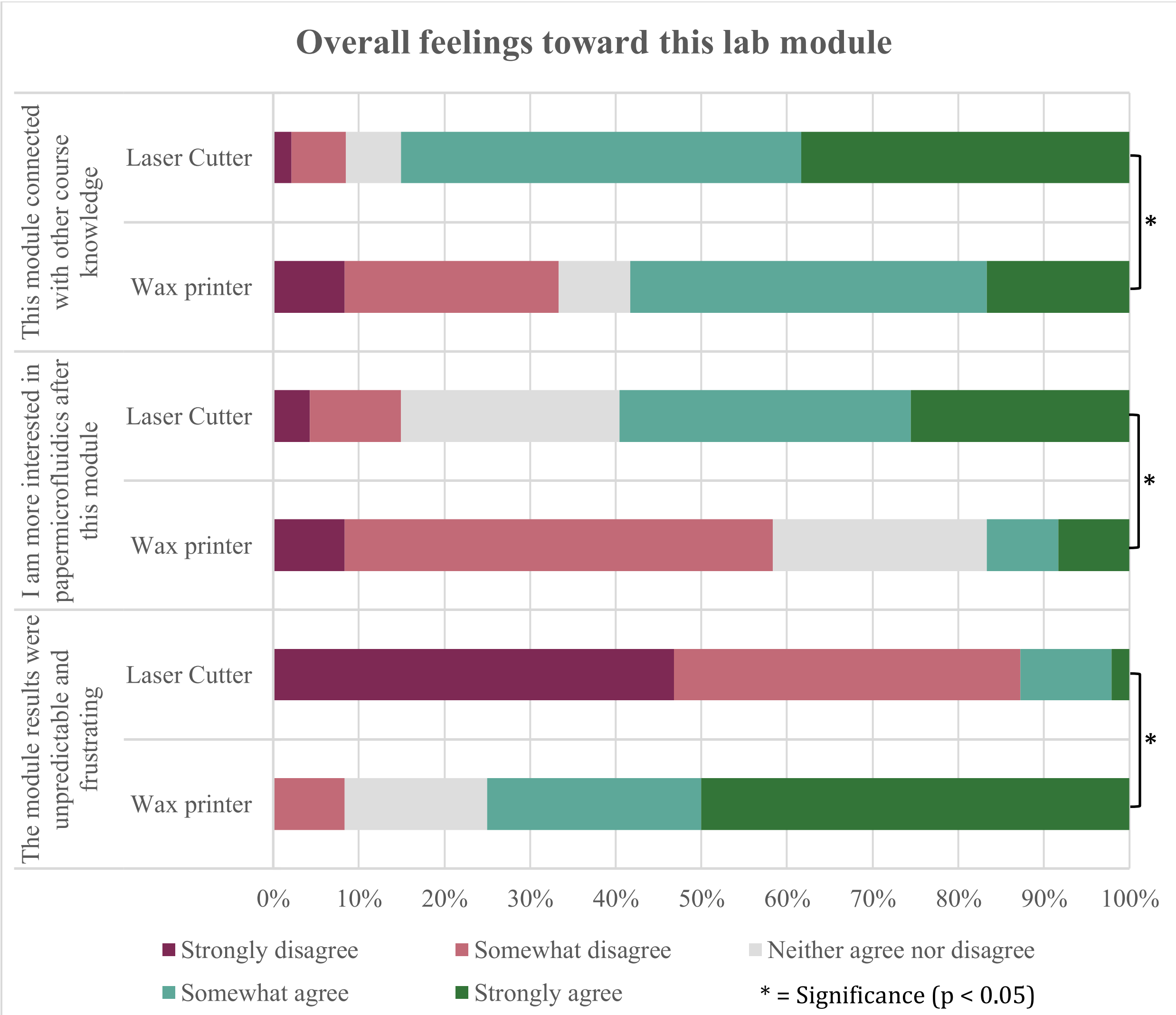

Figure 8: survey responses for questions regarding overall feelings toward paper microfluidic module (laser cutter responses = left, wax printer responses = right)

Figure 8 displays the survey results for questions regarding the overall feelings toward the laboratory module from students that used wax printers compared to laser cutters for fabrication. The feelings toward this lab module were consistent with the rest of the responses. Running a Wilcoxon rank sum test revealed that there was a significant difference found in the responses to each survey question regarding the overall feelings towards the lab. The percentage of students who felt that the results of the module were unpredictable and frustrating dropped from >70% of wax printer users, to <15% of laser cutter users. It was also found that the interest in paper microfluidics after completing the module increased in students who completed the module using a laser cutter.

Overall, students responded to the surveys agreeing that paper microfluidic chip fabrication was more consistent, feasible, and quicker with the laser cutter when compared to the wax printer.

Additionally, an understanding of paper microfluidic theory was maintained in the transition from wax printing to laser cutting. The practical application of paper microfluidics seemed to improve among laser cut paper microfluidic chips, with responses indicating that the experimental results were more consistent with theory than wax printed paper microfluidic chips. Finally, the responses also demonstrated that students who fabricated chips with a laser cutter felt better about the education they received throughout the module, with fewer responses indicating frustration at the module and more responses indicating further interest in the field.

Discussion

The results of this paper demonstrate an adherence to the Washburn equation and an overall improvement in the students' experiences with the lab module. There were a few limitations encountered with our current experimental setup that could be addressed in future iterations of this lab module. The narrow beam width of the laser, 0.05 mm, did not set the final minimum feature size of the paper channels due to the charring that would clog/hinder the flow of fluid. This limited the minimum width of the channels. This, along with the inherent transient period needed for the Washburn equation to take effect, placed restrictions on the minimum width and length of channels. Further experimentation with different papers or perhaps different power settings could help find a better fit for the lab module's purposes. The minimum channel width that worked was 1mm. Some future directions where the lab module could be taken include the application of Darcy flow, characterizing the flow of intersecting channels, and incorporating three-dimensional channels for more complex geometries.

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